REMARKS

Entry of the foregoing, reexamination and further and favorable reconsideration of the subject application in light of the following remarks, pursuant to and consistent with 37 C.F.R. § 1.112, are respectfully requested.

By the present amendment, claim 60 has been amended to further clarify Applicant's invention and new claim 65 has been added. Support for claim 65 can be found at least in prior claim 60. Accordingly, no new matter has been added by this amendment.

Turning now to the Official Action, the Examiner has rejected claims 46-64 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. Applicant respectfully traverses this rejection.

The Examiner has stated that it is unclear what Applicant intends to convey by the phrase "stable liposome" in the independent claims. The Examiner further has stated that the term "stable" is a relative term and that the stability of liposomes depends on the conditions to which they are exposed; such as time, light temperature, oxidants etc.

Applicant submits that the term "stable" in the context of liposomes loaded with a chemical species is well understood in the art to mean that an entrapped chemical species or the like remains inside the liposome for an amount of time after loading and after injecting the liposomes into an animal. In fact, for example, the stability of the liposomes with regard to leakage of the loaded chemical species is discussed on page 14, lines 5-19 of the

specification. Applicant states that:

> [a]fter incorporation the chemical will remain in the vesicle for fifteen minutes to several hours depending on the chemicals, until the buffer leaks out of the vesicle. One should be aware that decay of the initial drug content may occur because of dilution of the water volume outside of the vesicles when they are injected into an animal. This decay will generally occur much more slowly than the initial loading process because of favorable effects of the pH gradient on the vectorial movement of the drug across the vesical membrane. This insures that a drug will reach its targeted tissue before significant leakage out of the vesicles can occur. This time period of usually several hours allows the chemical or drug to be carried to its desired destination and prevents it from acting in areas that would be deleterious to the animal.

Furthermore, in Example 3 (page 18 of the specification), Applicant demonstrates that liposome vesicles loaded with a spin-labeled chemical in the presence of a pH gradient retained a much higher concentration of the spin-labeled chemical than liposomes loaded without a pH gradient. Therefore, liposomes loaded in the presence of a pH gradient had significantly less leakage and thus were more stable. In addition, Example 3 further indicates that it is not necessary to maintain the pH gradient after liposomes are loaded with a chemical species or drug. This feature of the present invention is important for preventing massive amounts of drugs from being dumped in the host upon administration of the liposomes.

One of the discoveries of the invention is that even though liposomes with pH or electrical potentials across their membranes could accumulate drugs with pH-responsive groups within their molecular structures or drugs having hydrophobic ions as part of their molecular structure, the loaded liposomes of the invention are stable and do not release their drugs upon being diluted into large volumes of body fluids.

With regard to claim 54, the Examiner is not clear as to what is conveyed by the phrase "hydrophobic ions." Applicant submits that the phrase "hydrophobic ion" refers to an ion that is surrounded by hydrophobic groups in its molecular structure to allow its partitioning into non-polar environments like the interior of lipid bilayer membranes. An example of such an ion is tetraphenyl phosphonium, which can be accumulated into liposomes, not necessarily having pH gradients, but having an electrical potential difference across their membranes. Many examples of hydrophobic ions are well known in the art.

The Examiner has further stated that the distinction between p-amino salicylic acid and salicylic acid derivatives in claim 60 is unclear since p-amino salicylic acid is a derivative of salicylic acid. In order to expedite prosecution in the subject application, and not to acquiesce to the Examiner's rejection, applicant has amended claim 60 to no longer recite p-amino salicylic acid. Claim 65 which is dependent upon claim 60 has been added so as to indicate that the salicylic acid derivative is p-amino salicylic acid.

In view of the above, withdrawal of the rejection under 35 U.S.C. § 112, second paragraph, is respectfully requested.

Claims 46-54, 56, 57, and 61-64 have been rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Nichols et al. (BBA, 1976). Applicant respectfully traverses this rejection.

Prior to addressing the merits of the Examiner's rejection, a brief description of applicant's invention is believed to be necessary. Essentially, the present invention is based on the surprising discovery that the use of pH gradients, in accordance with the invention, to

load liposomes allows for the rapid uptake of drugs or chemical species by the liposomes. In addition, the chemical-species loaded liposomes of the invention are stable in the presence of the pre-imposed pH gradient and in the absence of the pre-imposed pH gradient. This unexpected feature allows the liposomes to remain stably loaded after they are injected into a host where the pre-imposed pH gradient no longer exists. This feature of the present invention is important for preventing massive amounts of drug from leaking or being "dumped" in the host after administration of the drug loaded liposomes.

Applicant has experimentally shown that it is not necessary to maintain the pH gradient to keep the liposomes in a loaded state. Example 3 of the present application demonstrates that when the liposomes were placed in a ten-fold excess of lysine buffer, the pH gradient that had been preimposed for loading was largely collapsed, and as a result, very little leakage of the entrapped chemical occurred.

In contrast to the present invention, Nichols et al. teaches the uptake of catecholamine by liposomes maintaining a pH gradient. The liposomes of Nichols et al. remain loaded only in the presence of a pre-imposed pH gradient which suggests that upon administration to a host where the pre-imposed pH gradient no longer exists, massive amounts of catecholamine would leak from the liposomes into the host. In fact, Nichols et al. stated that "[w]hen the gradients were destroyed by ammonium chloride additions, the accumulated catecholamines were released, demonstrating that the uptake was reversible and dependent upon pH gradients." Further, according to Nichols et al., the accumulation of catecholamine occurred

slowly (loading was maximal at 90 minutes after addition of the amine), not rapidly as in the present invention.

Applicant respectfully submits that the subject invention is neither taught nor suggested by Nichols et al. This reference neither teaches nor suggests to one skilled in the art a method for the rapid preparation of stable drug or chemical species entrapped liposomes.

Nichols et al. discloses the physical chemistry involved in using a pH gradient to load catecholamines into a liposome. However, Nichols et al. does not teach or suggest that the loaded drug composition can be accumulated and entrapped within the liposome to produce a stable liposome vesicle-entrapped chemical species. In fact, Nichols et al. teaches that chemical species or drugs rapidly leak out of the liposomes when the pH gradient is destroyed suggesting that drug dumping will occur. This is in direct contrast to the teachings of the present application where it is not necessary to maintain a pH gradient to keep the drug or chemical species in the liposome after administration to the host. Upon reading the teachings of Nichols et al., the skilled artisan would in no way be led to use pH gradients in accordance with the invention to produce a stable liposome vesicle-entrapped chemical species. In fact, the skilled artisan could not produce liposomes in accordance with the teachings of Nichols et al. because the reference does not disclose the concentrations of citrate or phosphate necessary to form liposomes.

Further, Nichols et al. does not show a stable liposome, which retains the drug over a period of time. Nichols et al. does show the accumulation of catecholamine, i.e., loading,

over a period of 90 minutes, however, the reference does not show what happens after the liposome is loaded. Thus, it does not show that the liposome is stable.

Therefore, based on the foregoing, withdrawal of the § 102 rejection based on Nichols et al. is respectfully requested.

Claims 46-54, 57 and 61-64 have been rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Deamer et al. (BBA, 1972). Applicant respectfully traverses this rejection.

Like Nichols et al., Deamer et al. teaches the physical chemistry of pH gradients. However, there is no teaching that the physical chemistry of pH gradients could be used to accumulate and stably entrap a drug composition in liposomes. The primary focus of Deamer et al. is not to analyze loading liposomes with chemical species to form stable liposome vesicle-entrapped chemical-species, but rather to analyze the effects of a pH gradient on flourescent probes. Deamer et al. only uses liposomes to analyze the quenching effect on flourescent probes in the presence a pH gradient. In fact, in the discussion on page 334, Deamer et al. states that the liposome system offers a potential model system for studying the mechanism of electron transport across membranes. There is no suggestion or teaching in Deamer et al. that would lead the skilled artisan to a method of producing a stable liposome vesicle-entrapped chemical species

Therefore, based on the foregoing, the Examiner is respectfully requested to withdraw the § 102 rejection based on Deamer et al.

Claims 46-54, 59 and 61-64 have been rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Cramer et al. or Kano et al. Applicant respectfully traverses this rejection.

Cramer et al. also teaches the physical chemistry involved in using a pH gradient to load certain simple ionizable molecules (fumaric and maleic acid) into a liposome. Kano et al. teaches the use of trisodium 8-hydroxy-1,3,6-pyrene-trisulfonate, pyranine, as a probe for monitoring the pH in the interiors of negatively charged liposomes and at the outer surface of positively charged liposomes. Neither reference teaches or suggests the claimed invention of using a pH gradient to produce a stable liposome vesicle-entrapped chemical species.

Upon reading Cramer et al. or Kano et al., it would not have been clear to the skilled artisan whether the teachings of Cramer et al. or Kano et al. would work as a general mechanism to accumulate drugs and to produce stable drug-entrapped liposomes. Thus, those skilled in the art, even if they recognized the utility of the physical chemistry described by the references, would not have known whether the additional structural features present in drugs, such as multiple functional groups, would render the use of this physical chemistry workable for drug loading and entrapment. Nor would it have been obvious to one of ordinary skill in the art that a pH gradient could be used to prepare stable entrapped chemical compositions for therapeutic use as claimed by the present application.

The liposomes of Cramer et al. remain loaded only in the presence of a pH gradient which suggests that upon administration to a host, massive amounts of catecholamine would leak from the liposomes into the host. In fact, Cramer et al. states on page 299 (last line) that

"following a pH perturbation, an equilibrium condition, corresponding to zero net transport, should be reached where the internal and external H_2A activities are equal." In other words, if liposomes produced in the presence of a pre-imposed pH gradient are placed in an environment where the pre-imposed pH gradient no longer exists, massive amounts of H_2A would leak from the liposomes. Cramer et al. states in the last paragraph on page 300 that non-selective leakage of both the fumaric and maleic acid probably is the result of vesicle rupture in response to osmotic stress. These statements teach away from the present invention where drug entrapped liposomes remain stably loaded after they are injected into a host where the pre-imposed pH gradient used to load the liposomes no longer exists. Upon reading these references, the skilled artisan would not be lead to the present invention.

In view of the above, the Examiner is respectfully requested to withdraw the rejection under 35 U.S.C. § 102(b) over Cramer et al. or Kano et al..

Claims 46-64 have been rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Nichols et al., Deamer et al., Cramer et al. and Kano et al. Applicant respectfully traverses this rejection.

Applicant respectfully submits that the subject invention is not taught or suggested by any of the references cited by the Examiner. These references, taken alone or in combination, do not teach or suggest to one skilled in the art a method for the preparation of stable liposome vesicle-entrapped chemical species.

As stated above, one of the discoveries of the invention is that even though liposomes with pH or electrical potentials across their membranes could accumulate drugs with pH-

responsive groups within their molecular structures or drugs having hydrophobic ions as part of their molecular structure as taught by the references cited by the Examiner, the loaded liposomes of the invention are stable and do not release their drugs upon being diluted into large volumes of body fluids.

In addition to the comments set forth above regarding Nichols et al., Deamer et al., Cramer et al. and Kano et al., which are incorporated herein by reference, Applicant submits that experiments involving the injection of liposomes into a rat demonstrate the feasibility of the liposome drug delivery system *in vivo* (See Example 3 in the present application). Prior to these experiments, one would not have known whether such drug entrapped liposomes would wreak havoc on the biogenic amines that play a vital role in animal physiology. For example, as demonstrated in Deamer et al., catecholamines could be loaded into liposomes with pH gradients. However, until after Applicant's *in vivo* experiments were performed, no one could have predicted that an animal would tolerate a disturbance of its natural catecholamine status when such catecholamine-loaded liposomes were injected into its body. None of the cited references disclose information for *in vivo* administration of drug loaded liposomes.

Loading liposomes with drugs for therapeutic purposes was not obvious in view of Nichols et al., Deamer et al., Cramer et al. and Kano et al. There is no recognition that entrapped drug compositions could be obtained as claimed by Applicant and used therapeutically. In fact, prior to applicant's invention, many difficulties remained with liposomal carrier systems used for *in vivo* delivery of encapsulated drugs. *See, e.g.,* Mayer et

al., *Biochemica et Biophysica Acta*, 816:294-302 (June 27, 1985). Mayer et al. states that procedures for generating liposomes suffer significant drawbacks such as low trapping efficiencies, the presence of residual toxic agents (organic solvents and detergents), and time consuming protocols. Mayer et al. further states that before liposomes can be used to efficiently deliver drugs *in vivo*, a logical step-by-step system is required to be successful. In addition, Mayer et al. states that in the generation of an appropriate carrier system, one must demonstrate an ability to encapsulate and retain drugs of clinical interest in liposomes. Applicant has demonstrated the encapsulation and retention of drugs in liposomes.

Mayer et al. teaches the skilled artisan that the liposomes of the prior art possess many drawbacks and problems. Therefore, based on the teachings of the art cited by the Examiner (Nichols et al., Deamer et al., Cramer et al. and Kano et al.) and the teachings of Mayer et al., the skilled artisan would not expect to successfully produce a drug loaded liposome that would not leak or be toxic to the host. Only Applicant has shown in *in vivo* experiments that liposomes can be produced and administered to a host with no toxic effects.

Therefore, withdrawal of the rejection under 35 U.S.C. § 103(a) is respectfully requested.

From the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order and such action is earnestly solicited.

In the event that there are any questions relating to this Amendment, or the application in general, it would be appreciated if the Examiner would telephone the undersigned attorney concerning such questions so that prosecution may be expedited.

Respectfully submitted,

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